

Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*

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In the past decade much attention has focused on the role that genetics can play in the formation of management strategies in conservation. Here, we describe genetic diversity in the world's largest lizard, the Komodo dragon (*Varanus komodoensis*), examining the evolutionary relationships and population genetic history of the four islands in south-east Indonesia, which form the vast majority of its range. We identify distinct genetic groups for conservation. The population on the island of Komodo shows by far the largest values of genetic divergence and is proposed that it should be a separate conservation management unit. Other populations, surviving either on small islands with substantially reduced genetic variability, or in isolated patches, are identified as particularly vulnerable to stochastic threats and habitat loss. Our results provide an example of how data defining intraspecific levels of genetic divergence can provide information to help management plans, ensure the maintenance of genetic variability across populations and identify evolutionary potential within endangered species.

Keywords: population genetics; evolutionarily significant units (ESUs); microsatellites; reptiles; *Varanus komodoensis*

1. INTRODUCTION

The definition of evolutionarily significant units (ESUs) for conservation, introduced by Ryder (1986), has elicited discussions on the practical way of identifying such units (e.g. Pennock & Dimmick 1997; Waples 1998), and different designations have been proposed based on ecological, biogeographical, and morphological data to groups of individuals or populations (Waples 1991; Dizon *et al.* 1992; Vogler & DeSalle 1994; Legge *et al.* 1996). Although divergences in character-based features are important parameters to define ESUs, it has been suggested that conservation units should also consider the length of time the populations have been isolated, and that their identification should be based on molecular genetic data (Avice & Ball 1990; Moritz 1994a).

In particular, the identification of separate ESUs currently requires reciprocal monophyly of mitochondrial DNA (mtDNA) alleles and significant divergence of allele frequencies at nuclear loci. On the other hand, a less stringent definition identifies subdivided populations where divergence time has not been sufficient to accumulate evolutionarily diagnostic characters as management units (MUs). Based on this concept, different MUs are recognized by significant allele frequency differences regardless of the phylogenetic distinctiveness of the alleles (Moritz 1994b).

Using this approach, studies on the genetic structure of natural populations have been used to recognize different units for conservation in several taxa, such as marsupials (Moritz *et al.* 1996), fur seals (Lento *et al.* 1997) and fishes (e.g. Riddle *et al.* 1998).

However, there are examples where nuclear markers with relatively high levels of polymorphism and mutation rate, such as microsatellite or major histocompatibility loci, have provided a more sensitive indicator of population divergence than mtDNA (e.g. Pope *et al.* 1996; Hedrick & Parker 1998). ESUs have been defined on the basis of reciprocal monophyly assessed solely from differences at microsatellite allele frequencies (Small *et al.* 1998; Parker *et al.* 1999). Moreover, recent studies on ESU designation in endangered species using mtDNA have advocated the use of microsatellites to corroborate results and establish precise management guidelines (Moritz 1994b; Waits *et al.* 1998; Manceau *et al.* 1999).

In the present study, we describe the genetic divergence among populations of the Komodo dragon (*Varanus komodoensis*), an endangered species of monitor lizard endemic to five islands in the Lesser Sunda region, south-east Indonesia. Estimates of divergence time among four island populations and levels of gene flow calculated using Wright's statistics and Slatkin's methods have been described in Ciofi & Bruford (1999). Here, microsatellite analysis was applied to assess the phylogenetic distinctiveness and document the evolutionary relationships among five Komodo dragon populations sampled on four islands. We introduce a new statistical method to assess whether the pattern of gene frequencies observed among populations are best explained by a model of immigration-drift equilibrium

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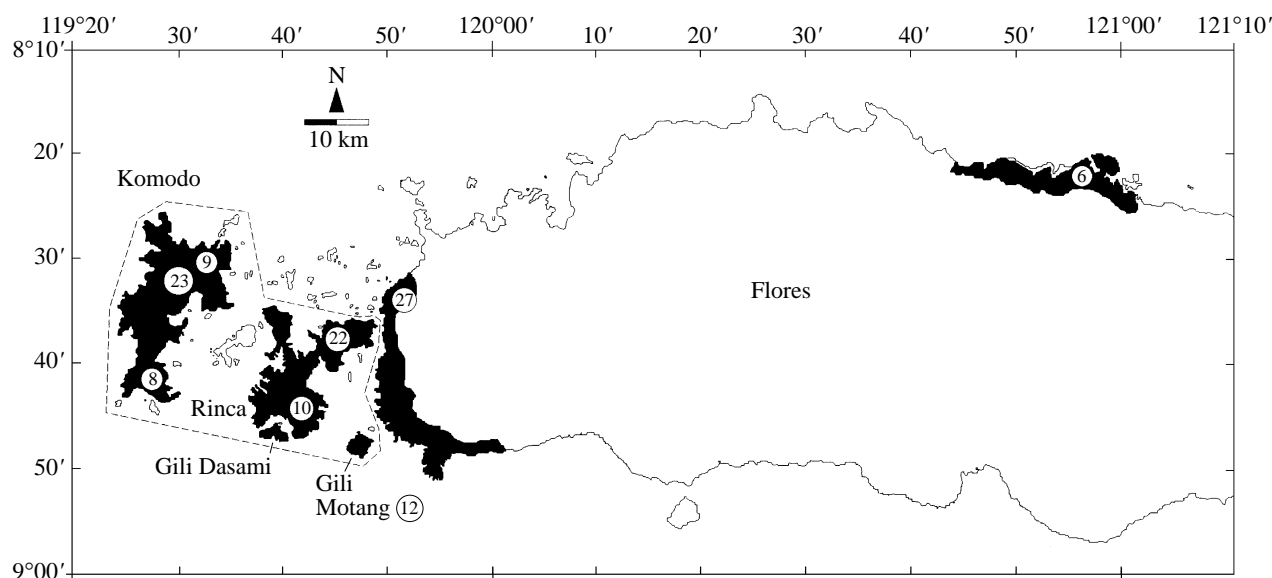


Figure 1. Map of the study site. Shaded areas show the known distribution of the Komodo dragon. The dashed line is the boundary of Komodo National Park. Numbers represent sample size for each sampling location.

or by a non-equilibrium model of fragmentation followed by drift with no gene-flow. Relating palaeogeographical data to genealogical history, we identify ESUs and provide information for management priorities.

2. METHODS

(a) Study sites and sample collection

The range of the Komodo dragon is confined to the islands of Komodo, Rinca, Gili Motang, Gili Dasami (part of Komodo National Park) and Flores (figure 1). The islands of Komodo, Rinca and Gili Motang contain a population of about 1600, 1100 and 100 animals, respectively (Komodo National Park, unpublished report), while a comprehensive survey on Gili Dasami and Flores has never been carried out. On Flores, extant populations are threatened by poaching of prey species (the Rusa deer *Cervus timorensis*) and habitat loss, which have substantially reduced the distribution of the Komodo dragon on the island in the last three decades (C. Ciofi and de Boer, unpublished data).

The study was conducted on the islands of Komodo, Rinca, Gili Motang and Flores, in 1994 and 1997. A total of 117 blood and tissue samples were analysed from individuals trapped in six locations on Komodo (40), Rinca (32) and Gili Motang (12), and in two different areas on the west (27) and north coast (six) of Flores (figure 1). Specimens were caught in baited traps (300 cm × 50 cm × 50 cm) as described by Ciofi (1999). Average weight, snout-vent length, and total length (mean ± s.e.) were 24.2 ± 2.8 kg (range: 0.6–69 kg), 96.1 ± 3.8 cm (range: 36–151 cm), and 206.7 ± 6.8 cm (range: 93–302 cm), respectively. Blood samples were obtained from the caudal vessels using a spinal needle (22 gauge × 8.9 cm) or by nail-clipping, and stored directly in a lysis buffer (Bruford *et al.* 1998) in order to preserve DNA at ambient temperature. About 0.5 g of tissue was also obtained by biopsy and subsequently preserved in 70% ethanol.

(b) Genetic analysis

Genetic diversity was assessed using ten polymorphic micro-satellite loci isolated from a genomic library enriched for di- and

trinucleotide repeats, as described in Ciofi & Bruford (1998). Allelic frequencies, and mean observed and unbiased expected heterozygosity (Nei 1987) were calculated using GENEPOP (Raymond & Rousset 1995) version 3.1b. The F_{ST} estimator θ (Weir & Cockerham 1984) was calculated using GENETIX 3.07 (Belkhir *et al.* 1996–1998) to assess genetic differentiation among populations. Statistical significance of θ was tested using 1000 permutations.

The degree of genetic divergence among populations was estimated using $(\delta\mu)^2$, calculated as the mean squared difference between allele sizes (Goldstein *et al.* 1995), and Nei's standard distance D , a measure of the proportion of pairwise comparisons in which two alleles drawn from two populations are different (Nei 1987). The percentage of shared alleles between populations was also estimated. All values were obtained using the program MICROSAT (Minch *et al.* 1995–1997), v. 1.5d.

Cluster analysis was carried out using the genetic distances computed for all pairs of populations using the neighbour-joining clustering algorithm implemented in the program NEIGHBOR of the software package PHYLIP (Felsenstein 1993). The significance of a particular topology was estimated with 1000 bootstrap replicates and a consensus tree was then constructed using the program CONSENSE.

(c) Population structure models

The genealogical history of alleles among islands was inferred considering two models of population structure. The first (gene flow model) assumes that the gene frequencies within islands are determined by a balance between genetic drift and immigration. In the second (drift) model, it is assumed that an ancestral panmictic population separated into several independent units which start diverging purely by genetic drift. Both models assume that the effects of micro-satellite mutations are negligible. Specifically, it is assumed that the mutation rate is much smaller than the immigration rate in the gene flow model, and that the reciprocal of the mutation rate is much longer than the divergence time in the drift model. This was considered reasonable for the Rinca, Flores, and Gili Motang populations, because of the lack of

Table 1. Nuclear genetic variation in Komodo dragons per population for all loci combined

(A , mean number of alleles; H_O , mean observed heterozygosity; H_E , mean expected heterozygosity. All values with standard errors.)

	sample size	A	H_O	H_E
Komodo	40	3.70 ± 0.80	0.27 ± 0.08	0.30 ± 0.09
Rinca	32	3.10 ± 0.62	0.33 ± 0.09	0.37 ± 0.08
Flores west	27	3.70 ± 0.79	0.48 ± 0.06	0.48 ± 0.06
Flores north	6	2.60 ± 0.34	0.66 ± 0.11	0.56 ± 0.06
Gili Motang	12	1.80 ± 0.25	0.17 ± 0.07	0.23 ± 0.08

Table 2. Proportion of shared alleles (below diagonal) and θ -values (above diagonal) between Komodo dragon populations for ten microsatellite loci

	Komodo	Rinca	Flores west	Flores north	Gili Motang
Komodo	—	0.394	0.357	0.559	0.458
Rinca	0.45	—	0.073	0.447	0.249
Flores west	0.36	0.77	—	0.254	0.203
Flores north	0.19	0.43	0.56	—	0.559
Gili Motang	0.44	0.64	0.60	0.34	—

Gene flow depends on Nm , where N is the population size and m is the immigration rate. The amount of drift depends on t/N , where t is the time of divergence. The method calculates the relative likelihood of the two demographic models, given the data, averaged over all values of t/N or Nm weighed by their likelihood. Therefore, no assumption need to be made on Nm or t/N . Using this method it is also possible to estimate t/N or Nm conditional on their respective models.

Our method is based on the comparison of the likelihoods for the two models, given the observed microsatellite frequency counts (i.e. numbers of chromosomes belonging to each length category) using coalescent theory and Markov Chain Monte Carlo simulation (see the electronic appendix on the Royal Society Web site). The method takes into account both sampling and genealogical variation in gene frequencies.

3. RESULTS AND DISCUSSION

The average number of alleles per locus across all populations for the ten microsatellites studied was 3.25 ± 0.33 (range: 2–14). The highest and lowest number of alleles was observed for the island of Flores and Gili Motang, respectively. Expected heterozygosity ranged from 23% in Gili Motang, to 56% in the population sampled on the north coast of Flores (table 1).

A high degree of variation was detected among the populations of Komodo, Rinca, Gili Motang, west and north Flores. Analysis of allele frequencies revealed high levels of heterogeneity ($\theta = 0.369$, $p < 0.001$). Significant differences in allele frequency distributions (figure 2) and θ -values were also found for all pairwise comparisons between populations ($p < 0.01$). A lower, but still significant value of θ was recorded in the comparison between the sample from west Flores and the population of Rinca (table 2), which shared the highest proportion of alleles (77%).

(a) Molecular phylogeny and ESU designation

Overall, genetic divergence was most marked for the Komodo island population, which had eight unique (private) alleles and consistently very high and significant F_{ST} values (range: 0.32–0.68, $p < 0.001$). Moreover, genetic distance measures involving Komodo were consistently the highest among all comparisons. Unrooted neighbour-joining trees were built based on both Nei's D and Goldstein's $(\delta\mu)^2$. Although significant differences ($p < 0.05$) were found in the comparison among individuals sampled in three different locations on Komodo (see figure 1), the sampling sites always cluster together within dendrograms constructed to indicate the affinities

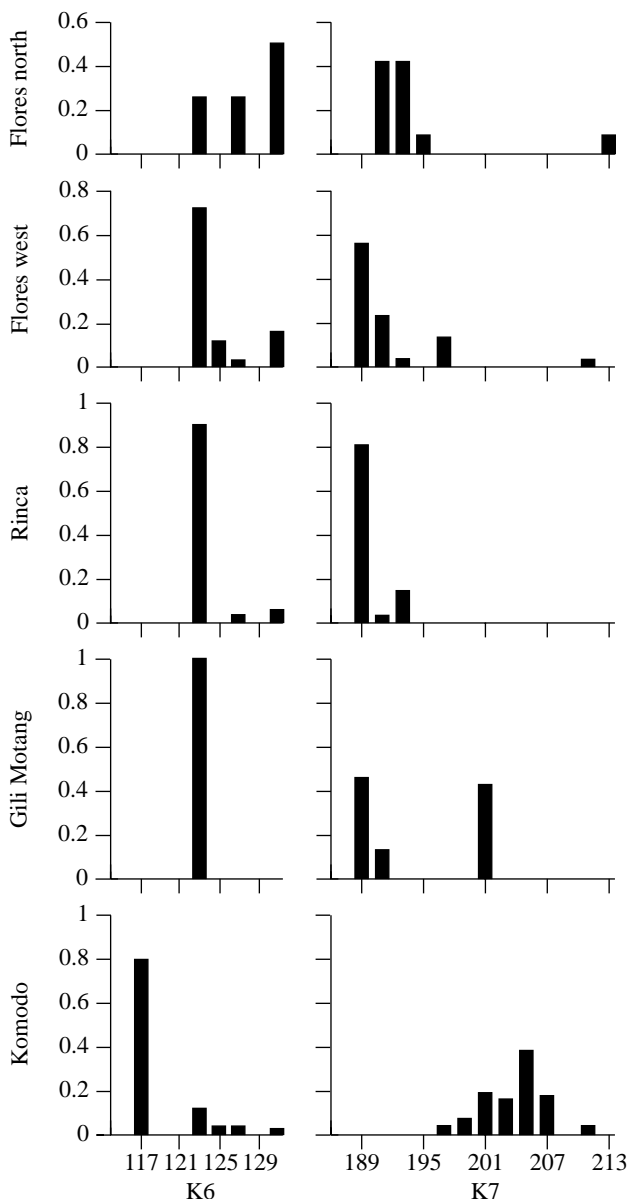


Figure 2. Allele frequency distribution of two microsatellite loci in five populations of Komodo dragons. Numbers for each locus indicate allele sizes in base pairs.

substantial barriers to gene flow compared with the Komodo population, and the possibility of recent foundation of the Gili Motang population and fragmentation of the Flores populations.

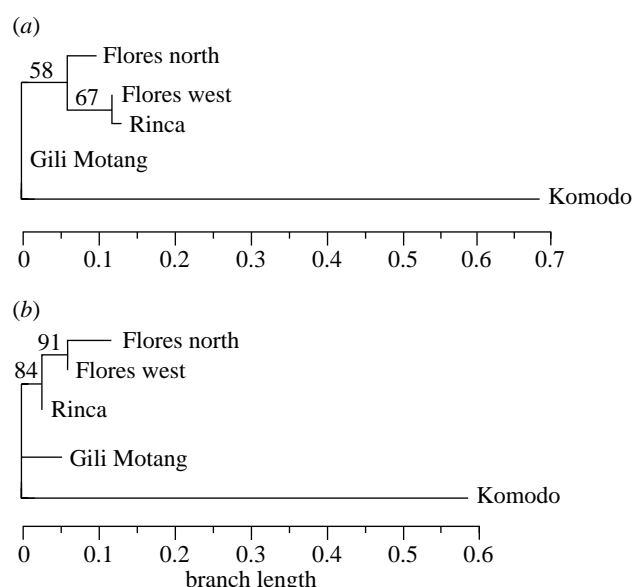


Figure 3. Unrooted neighbour-joining trees of Komodo dragons based on (a) Goldstein's $(\delta\mu)^2$ values and (b) Nei's standard genetic distance D . Branch lengths refer to the values calculated by the neighbour-joining algorithm based on the pairwise comparison matrix of genetic distances. Numbers above branches indicate the percentage of 1000 bootstraps in which the cluster to the right was present.

among populations. The most noticeable result from these analyses was the marked separation of Komodo from Rinca, Flores and Gili Motang (figure 3).

A different palaeogeography also distinguishes Komodo from the other islands. According to eustatic sea level variations in the past 140 kyr (Chappell & Shackleton 1986; McCulloch *et al.* 1999) and bathymetric data of the study area, Flores and Rinca separated for a brief period during a high sea level event about 125 kyr before present (BP) and were then connected to each other until 10 kyr BP. While Gili Motang was connected several times to Flores and Rinca, Komodo was subject to a long period of isolation, and appears to have been joined with the eastern islands only 140 kyr BP and 18 kyr BP, during the last two Pleistocene glacial maxima. This association may have lasted up to 20 kyr and about 6 kyr in the first and second period, respectively (Chappel & Shackleton 1986). Estimates of divergence time for Komodo island (see Ciofi & Bruford 1999) suggest that immigration may have occurred between Komodo and the other islands during the last two glaciations. Afterwards, the rise of the sea level and the consequent increased distance to Rinca might have prevented movements to and from Komodo, despite the ability of Komodo dragons to swim (Auffenberg 1981).

The historical isolation and genetic distinctiveness of Komodo highlight the conservation value of this population from both a management and evolutionary perspective. The degree of allelic diversity of Komodo island and its consistent separation from all other populations in cluster analysis, indicates that it should certainly be regarded as a separate MU, and most probably as an ESU.

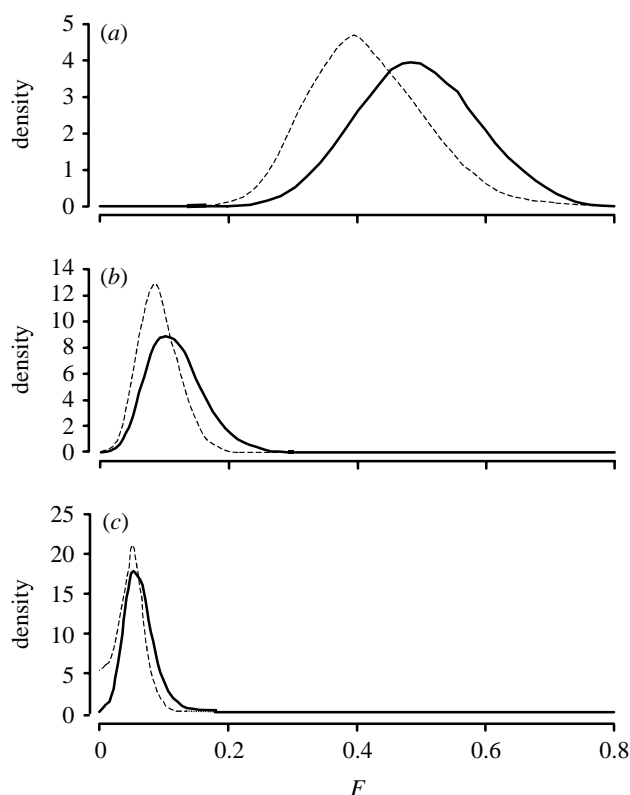


Figure 4. The marginal posterior density for F under the two different models is plotted for the populations of (a) Gili Motang, (b) Rinca and (c) Flores west. The solid line shows the distribution under the gene flow model, and the dotted line shows the distribution under the drift-divergence model.

In practice, both ESUs and MUs have the same conservation management implications: different units should, if possible, be managed separately. Komodo is clearly of some importance in this respect. Considering the estimated size of about 1600 individuals, larger than Rinca and more than an order of magnitude higher than Gili Motang, the population of Komodo is of considerable significance for maintaining both a substantial level of genetic variability within the species and any adaptive differences which may quickly arise between islands (Losos *et al.* 1998).

(b) *Genealogical history and population management*

The pattern of genetic divergence among Rinca, Gili Motang and the two populations on Flores was not as pronounced as for Komodo. Gili Motang showed significantly different allele frequencies ($p < 0.001$) from the other populations and was clearly separated in the cluster analysis. However, it did not possess a suite of unique alleles which might indicate a distinct evolutionary history. On Flores, on the other hand, the sample from the north coast had three private alleles, and was significantly different ($p < 0.05$) from the west coast. Nevertheless, the cluster analyses produced different topologies for the Flores populations (figure 3), making their phylogenetic relationship difficult to interpret.

Thus, while the genetic characteristics and palaeogeography of Komodo provide adequate information for setting conservation priorities, decisions for the

management of the other island populations of Komodo dragons are better made by inferring the genealogical history of alleles since the divergence of the populations occurred.

Between the two models of population structure described in this study, the likelihood of the gene flow model was six times higher than that of the drift model ($p(\text{gene flow model}) = 0.85 \pm 0.004$; Bayes factor = 6) when Rinca, Gili Motang and Flores west were considered. The extent of the interaction between drift and gene flow was estimated by looking at F (the probability that two genes share a common ancestor within a population), and M (the number of migrants per generation), calculated from the F values (see the electronic appendix on the Royal Society Web site). A high level of immigration relative to drift was inferred in Flores west ($F = 0.04$, 90% highest posterior density (HPD) range: 0.002–0.07; $M = 5.5$, 90% HPD range: 3.1–421.3) and Rinca ($F = 0.09$, 90% HPD range: 0.04–0.19; $M = 2.3$, 90% HPD range: 0.72–13.1), while on the small island of Gili Motang immigration had a smaller effect relative to drift ($F = 0.49$, 90% HPD range: 0.34–0.67; $M = 0.25$, 90% HPD range: 0.13–0.53) (figure 4). Using information available from captive breeding programmes and wild populations (Auffenberg 1981; Seal *et al.* 1995), we considered a generation time for Komodo dragons of about 12 years. Using this estimate, one migrant every 60 years would have reached Gili Motang since the split from Flores and Rinca. Although our analysis does not advocate a separate evolutionary history for Gili Motang, such a low level of gene flow is not sufficient to balance the effect of drift on the genetic divergence of this population, neither is it likely to be sufficient to counteract the effect that small population size, low heterozygosity and high percentage of fixed alleles may have on population viability (O'Brien & Evermann 1988; Mills & Allendorf 1996; Saccheri *et al.* 1998).

The gene flow model had also a high likelihood ($p(\text{gene flow model}) = 0.890.002$; Bayes factor = 9) in the comparison between north and west Flores. The effect of genetic drift on allelic differentiation was more evident on the north ($F = 0.31$, 90% HPD range: 0.16–0.49; $M = 0.56$, 90% HPD range: 0.17–2.73) than on the west coast of the island ($F = 0.04$, 90% HPD range: 0.00–0.19; $M = 6.0$, 90% HPD range: 1.9–250). This result indicates a gradual fragmentation of the species on Flores. This process presumably started about 8 kyr BP, with the onset of agriculture in south-eastern Indonesia (Monk *et al.* 1997). The genetic differentiation of north Flores suggests an historically low level of gene exchange. However, the high likelihood of the gene flow model indicates that the present isolation between north and west Flores is a relatively recent event, highlighting the effect of increasing human activity on the survival of the species on this island.

Under this scenario, isolated populations such as those on Gili Motang and Flores should be monitored systematically and considered as an important part of genetic management plans. Future augmentation of the population of Gili Motang should consider Rinca or west Flores as possible sources, while management plans on Flores should incorporate gene flow between extant populations.

4. CONCLUSIONS

Although habitat protection is undoubtedly a priority and demographic studies should also be implemented in the effort to conserve Komodo dragons and determine population viability, our results show how population genetics can provide important information for the *in situ* management of this unique animal. The relevance of genetic analyses is highlighted here for the short-term management of vulnerable populations, such as those on Flores and Gili Motang. Moreover, we emphasize the importance of the identification and protection of populations with distinct evolutionary potential, such as that of Komodo, which is crucial for the maintenance of genetic components of biodiversity within this species.

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An electronic appendix to the paper can be found at (http://www.pubs.royalsoc.ac.uk/publish/pro_bs/rpbl435.htm).